

Vaginal pH and microflora related to yeast infections and treatment

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SUMMARY The relationship between vaginal pH, microflora, and yeast infection was investigated in 93 women randomly treated with either nystatin or miconazole pessaries and cream for two weeks. The vaginal pH was measured in a control group of 48 women. In the study group, 37 patients defaulted, 39 were cured, and 17 required treatment during the six-month follow-up period. In both study and control groups before and after treatment the mean vaginal pH was in the range of 4.3-4.6. Lactobacilli were plentiful in 78 (91%) out of 86 patients and shows that lactobacilli and yeasts commonly coexist. The influence of other organisms appeared to be negligible. The trial showed that nystatin and miconazole were equally effective in the treatment of vaginal yeast infection and that the broad-spectrum activity of miconazole offered no advantage in this condition.

Introduction

Several previous reports have been published concerning the relationship between vaginal yeasts, other microflora, and their effects on pH. Peeters and his co-workers¹ found a higher pH (in the lateral fornix) in patients with clinical signs or symptoms of candidal vaginitis than in controls, but not all patients had the diagnosis confirmed by culture. They also concluded that there was no correlation between pH, symptoms, and the number of colonies of pathogens, but an inverse relationship between the presence of candida and lactobacilli was found. Cohen² demonstrated a lower pH in the posterior fornix in women with clinical vaginitis than in controls. Both studies included some pregnant women and women with other vaginal pathogens and are therefore not directly comparable, but they do indicate conflicting results concerning vaginal pH and the presence of yeasts.

In the present study, our aim was to monitor the changes in pH values in a group of women with yeast infection in response to treatment. We also examined changes in pH values in relation to the menstrual cycle and the use of oral contraceptives. At the same time we assessed the vaginal flora and its changes in response to therapy with the standard antifungal

agent, nystatin, and a newer broad-spectrum imidazole derivative, miconazole, with activity against a wide range of fungi and some Gram-positive bacteria.

Patients and methods

STUDY GROUP

The study group consisted of 93 women attending the Department of Genitourinary Medicine at the West London Hospital who had symptoms of vaginitis, or whose contacts had balanitis, and who had yeast cells or hyphae detected on a Gram-stained smear. Details of last sexual intercourse, last menstrual period, method of contraception, recent treatment, parity, contact's symptoms, and relevant past history were all recorded. Symptoms and signs, together with the pH value and the results of smears and cultures for *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and yeasts, were noted. Samples were taken on each occasion for culture of lactobacilli and other vaginal micro-organisms.

After diagnosis by microscopy the patients were treated randomly with either nystatin two pessaries at night for two weeks, with cream twice daily, or miconazole one pessary at night for two weeks, with cream twice daily. These treatments were pre-packaged in plain coded envelopes so that the prescriber did not know which treatment was given. The patients were asked to attend monthly for three months for follow up and thereafter if symptoms returned.

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CONTROL GROUP

The control group was composed of a random sample of 48 patients of similar age, country of origin, and contraceptive practice attending the same department and who were free of genital infection.

MEASUREMENT OF pH

Vaginal pH was measured using a Corning-Eel digital pH meter with a combination electrode with semi-micro tip (requiring only 0.3 ml of fluid). The electrodes were calibrated for each measurement using two buffers, pH 7.0 and pH 4.01, between which they were rinsed thoroughly with deionised water. Various techniques for obtaining the measurements were tried, but the most stable reproducible pH readings were obtained by inserting the electrode directly into the vagina to a distance of approximately 4 cm. By this method the pH reading stabilised within 5-10 seconds. In each case the average of three readings to two decimal places was obtained. The electrodes were washed with deionised water between recordings. After each patient had been tested the electrode was cleaned under running tap water and placed in 2% glutaraldehyde solution for at least 30 minutes; the electrodes were then immersed in pH 7 buffer until required again. All recordings of buffer were made at room temperature and the meter was adjusted to 37°C for vaginal readings.

MICROBIOLOGY

To exclude the principal pathogens the following investigations were carried out:

N gonorrhoeae

Gram-stained smears from the cervix and urethra were examined for typical Gram-negative intracellular diplococci. Samples were inoculated directly on to "split" plates containing a modified Thayer-Martin medium in one half and the same base without antibiotics in the other. The plates were incubated immediately for 48 hours at 37°C in candle-extinction jars. *N gonorrhoeae* was identified on culture by oxidase reaction and microscopical appearance in Gram-stained films. Confirmation was by immunofluorescent staining.³

T vaginalis

Direct examination of a wet preparation of vaginal secretion was accompanied by culture in Feinberg-Whittington medium and examined after 48 hours' incubation at 37°C.

Yeasts

A Gram-stained smear of vaginal material was examined and cultures made on Sabouraud's agar.

Cultures were incubated for 48 hours at 37°C and any yeasts isolated were identified by (1) germ-tube production; (2) chlamydospore formation on corn meal agar; (3) typical colonial appearance on tetrazolium agar; and (4) sugar fermentation tests.

Other micro-organisms

A high vaginal swab was taken and sent to the laboratory in Stuart's transport medium. The following culture plates were inoculated: 2×5% horse blood agar; MacConkey agar; Rogosa agar (Difco); and mycoplasma agar (base and supplement, BBL). The cultures were incubated aerobically for 48 hours at 37°C except one blood agar plate, which was placed in an anaerobic environment. The mycoplasma plates were incubated in 10% CO₂ for five days.

Ureaplasmas and chlamydia were not looked for. *Corynebacterium vaginale* was not specifically identified. The term, Gram-variable cocco-bacilli, was used to describe organisms of similar morphology.

Organisms were identified according to standard methods.⁴ Lactobacilli were detected by typical growth on Rogosa agar.⁵

An additional smear of vaginal material was Gram-stained and examined later for yeasts in all those cases in which the initial positive smear result was not supported by a positive yeast culture result. Lactobacilli were also looked for in all cases with negative culture results on Rogosa agar.

ANALYSIS OF DATA

The response to treatment was analysed by the χ^2 test with Yates's correction.

Results

STUDY GROUP

The 93 women were aged between 15 and 49 years (mean 24.5 years). A past history of yeast infection was obtained in 50 (54%). In 25 (27%) a penile irritation or rash was present in a sexual partner. Eighty-nine women (97%) were symptomatic and 88 (95%) had a vulval itch or discharge. Oral contraception was used by 52 (56%) women. Presentation for treatment at the clinic in relation to the menstrual cycle is shown in Table 1.

TABLE 1 *Diagnosis in relation to menstrual cycle*

	Week of menstrual cycle				Total
	1	2	3	4	
No of patients with yeast infection*	13	25	25	26	89

*Two patients were post-menopausal and two had amenorrhoea

The mean pH of the study group was 4.6 (Table II). No patient was menstruating at the time of diagnosis. Three patients had trichomoniasis and one gonorrhoea; there was only one woman whose partner had non-specific urethritis and she also had trichomonal infection both before and after initial treatment for yeast infection. In the study group, neither the outcome of treatment nor the drug given was related to, or had an effect on, vaginal pH (Table III).

TABLE II pH values for study and control group of patients

Patient group	Total No of patients	Range of pH							Mean
		3.5-3.9	4.0-4.4	4.5-4.9	5.0-5.4	5.5-5.9	6.0-6.4	6.5-6.9	
Study	93	9	39	29	7	6	2	1	4.6
Control	48	4	23	7	7	6	1		4.6

TABLE III pH values of study group related to treatment

Study group	Mean pH	No	%
Initial pH	4.6	93	100
Cured patients' initial pH	4.6	39	42
Cured patients after treatment	4.3	39	42
Relapsing patients' initial pH	4.5	17	18
Relapsing patients at relapse	4.4	13	14
Nystatin-treated patients at 1 month	4.1	16	17
Miconazole-treated patients at 1 month	4.3	12	13

CONTROL GROUP

The 48 control patients were aged 18-45 years (mean 25.4 years). The mean pH was 4.6 (Table II). No patient was menstruating at the time of examination. None was infected with yeasts, trichomonads, or gonococci; three patients were sexual contacts of men with non-specific urethritis. No variation in pH was found throughout the menstrual cycle and there was no relationship with oral contraception (Table IV).

TREATMENT (STUDY GROUP)

The outcome of treatment is shown in Table V. Vaginal infection recurred within six months in 11 (12%) patients, who were treated with nystatin and in

TABLE IV pH values of control group related to week of menstrual cycle and contraception

Menstrual cycle (week) and contraception	Mean pH	No	%
1	4.7	11	23
2	4.5	11	23
3	4.6	12	25
4	4.7	14	29
Contraceptive pill	4.6	31	65
Others	4.6	17	35

TABLE V Outcome of treatment

Treatment	No of patients						Total
	Defaulted*	Cured†	Relapsed (months)				
			1	2	3	4	
Nystatin	15	21	2	5	2	2	47
Miconazole	22	18	1	4	1	0	46
Total	37	39	3	9	3	2	93

*No follow-up test

†At least one negative culture result

6 (6%) patients, who were treated with miconazole (χ^2 0.213, $P=0.5$). At the first follow-up visit, two weeks after completion of treatment, only three patients had relapsed, two treated with nystatin and one treated with miconazole.

MICROBIOLOGY (STUDY GROUP)

The yeasts were identified as *Candida albicans* in 91 patients. One patient was colonised by *Torulopsis glabrata* and one by *Candida parapsilosis*.

Samples for extended microbiological studies were received from 86 patients at the initial visit. Follow-up samples were received from 52 patients—37 on one occasion, 11 on two occasions, and four at three follow-up visits.

The relationship in pre-treatment samples between vaginal pH values, presence of lactobacilli, and other organisms is shown in Table VI. In addition to lactobacilli, the other organisms isolated were:

Bacteroides species
Bacillus coli
 β -haemolytic streptococci
 Diphtheroids
 Faecal streptococci
 Gram-variable cocco-bacilli
 Klebsiella species
 Mycoplasma species
 Non-haemolytic streptococci
 Proteus species
Staphylococcus aureus
Staphylococcus epidermidis

TABLE VI Relationship between pre-treatment vaginal pH values and presence of lactobacilli and other bacteria on culture

	Mean (range) pH values		
	4.2 (3.9-4.7)	4.7 (3.7-6.5)	4.9 (4.2-5.8)
No of patients	23	55	8
Culture results			
Lactobacilli	Abundant	Moderate	Absent or very scanty
Other organisms	Scanty	Abundant	Abundant

The distribution throughout the samples was variable. No predominant organism was found in specimens with scanty or no lactobacilli, but follow-up specimens from this group showed the recurrence of lactobacilli in three of four patients investigated after treatment.

No notable change in microflora was found after treatment in the other groups examined.

Discussion

The question of the relationship between vaginal pH values and morbidity due to *C albicans* and other infections has produced conflicting reports with regard to the benefit of a low vaginal pH. Our work has indicated no relationship between vaginal pH over the range observed in 93 patients and 48 controls (pH 3.6-6.5) and vaginal morbidity due to yeast infection. Thus the protective effect of vaginal acidity does not appear to be confined to a critical range above or below which there is increased susceptibility to vaginal infection.

A relationship between the numbers of lactobacilli and vaginal acidity, however, is clearly shown and is consistent with the physiological role of the organism in maintaining a low pH in the vagina. Moreover, it appears that the part played by other organisms in the vagina rarely assumes importance, even when the pH value is at the higher end of the range observed. Increasing dominance of any organism normally considered commensal may lead to an inflammatory

vaginal reaction, but this did not occur in our experience among patients with candidal infection.

Treatment with both drugs was highly effective in the short term, with mycological cure in all but three who returned for follow up at one month (a cure rate of 91.3% with nystatin and of 94.7% with miconazole). Comparison of cure rates subsequently is increasingly invalidated by an inability to distinguish treatment failure from reinfection with further yeasts.

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